PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of

Yuji UENO, et al.

Docket No: Q107169

Appln. No.: 10/574,016

Group Art Unit: 1644

Confirmation No.: 4347

Examiner: KIM, YUNSOO

Filed: March 29, 2006

For: METHOD OF STABILIZING ANTIBODY AND STABILIZED SOLUTION-TYPE ANTIBODY PREPARATION

DECLARATION UNDER 37 C.F.R. § 1.132

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Dr. Toshihito Hosokawa, hereby declare and state:

THAT I am a citizen of Japan. I have graduated with Master of Engineering degree from Osaka City University, Osaka, Japan in 1989. I have worked at Drug Formulation Research and Development Laboratories, Kyowa Hakko Kirin Co., Ltd. since 1989. At Drug Formulation Research and Development Laboratories, I have been working on the formulation study and process development for developing drug products. In 2002 and 2003, I have joined a collaborative research program with Professor David J. W. Grant (Department of Pharmaceutics, College of Pharmacy, University of Minnesota, MN, USA) in order to learn the physico-chemical properties of solid state drug substances. I got Ph.D. degree of Pharmaceutical Sciences from Kyoto University in 2004. I belong to the Pharmaceutical Society of Japan for 19 years. I had several presentations in the academic meetings of this and other international societies, such as the International Conference of Controlled Release Society. My publications in academic journals are more than ten; these journals include the

Journal of the American Chemical Society, Crystal Growth and Design (published by American Chemical Society), and Biological and Pharmaceutical Bulletin (published by the Pharmaceutical Society of Japan) etc;

THAT I am familiar with the prosecution history of the above-identified application. I carefully reviewed the Office Action dated January 30, 2009, issued in the instant application, and am familiar with the rejection of Claims 14-21 under 35 U.S.C. §103(a) as allegedly being unpatentable over EP 1174148 in view of U.S. Patent Application Pregrant Publication No. 2003/0190316.

The present Declaration is submitted in support of Applicants' position that the presently claimed composition possesses properties that would not have been expected by one of ordinary skill in the art, and which are of clear practical significance for the preservation of antibody titer in stabilized antibody solutions, and for safety upon *in vivo* administration of the antibody solution.

The following experiments were conducted to demonstrate the criticality of glycine *and* citric acid in preventing the formation of soluble associations of antibody, which in addition to reducing titer, increase the likelihood of fever, nausea or hypotension, upon antibody administration to a patient.

I. Preparation of glycine-stabilized buffer

An acidic glycine-stabilized buffer was prepared in the manner described in Examples 1 and 2 of the specification. This glycine buffer (herein "Formulation A") consisted of an aqueous solution of glycine to a concentration of 23mg/ml, at pH 5. To determine antibody stability in this solution, the same antibody was used as described in Example 1 of the

specification, namely KM-871, a human chimeric antibody that specifically binds ganglioside GD3. Formulations B, C and D herein represent "Formulation 2," "Formulation 4," and "Formulation 1" in Table 1 of the specification as filed, respectively. The compositions of Formulations A-D are shown in the following table, Table 1:

Table 1

	KM-871 Antibody Concentration (mg/mL)	Additive	pH
Formulation A	5	Glycine: 23 mg/mL	5
Formulation B	2	Citric acid: 10 mmol/L	6
Formulation C	2	Glycine: 23 mg/mL Citric acid: 10 mmol/L	6
Formulation D	2	Phosphoric acid: 10 mmol/L	6

II. Antibody Stability in Formulations A-D

Following the exact experimental procedure used in Example 2 of the specification as filed, Formulation A was incubated at 40°C for 1 month, at which time the amount of soluble associations of antibody, and the amount of chemically degraded product, were assessed by HPLC gel filtration. The increase in soluble associations of antibody, and the amount of chemically degraded product, were calculated by subtracting the initial value from the measurement value. The amount of soluble associations of antibody, and the amount of chemically degraded product, are shown in the following table, Table 2. The data for Formulations B-D are from Table 3 of the specification as filed.

Table 2

The amount of increase during storage of 40°C for 1 month		
	Soluble associations (%)	Chemically degraded products (%)

Formulation A	0.18	0.55
Formulation B	0.20	0.59
Formulation C	0.02	0.51
Formulation D	0.20	1.48

As depicted in Table 2 above, an antibody solution buffered with glycine alone (*i.e.*, Formulation A), or an antibody solution buffered with citric acid alone (*i.e.*, Formulation B) results in an increase in soluble associations of antibody of 0.18% and 0.20%, respectively, after incubation at 40°C for 1 month. Phosphoric acid (*i.e.*, Formulation D), an art-recognized acidic buffer for antibody stabilization, produces a comparable increase in soluble associations of antibody (*i.e.*, 0.20%). In contrast, when citric acid and glycine are combined in the same buffer (*i.e.*, Formulation C), the increase in soluble associations of antibody is only 0.02%. That is, when glycine and citric acid are combined, a synergistic effect in suppressing the formation of soluble associations of antibody is observed, such that the amount of soluble associations is ten-fold less than when either citric acid or glycine is present alone. This result would not have been expected nor predicted by one of ordinary skill in the art at the time of the invention.

I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: May 25, 2009 Toshihito Hasegawa